STEM CELL THERAPY FOR MYOCARDIAL REPAIR

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Ischaemic myocardial damage is an increasing cause of heart failure in the western world and has long been considered irreversible because adult cardiomyocytes are terminally differentiated and do not proliferate.[w1] Reversal of heart failure would require replacement of damaged myocytes and restoration of blood flow. Over recent years attention has turned to the potential of stem cells to repair damaged hearts because of their ability to differentiate in vitro into cardiomyocytes, endothelial cells, and pericytes. The aim of this article is to outline the basic scientific principles of stem cell biology, to review the evidence for myocyte and vascular regeneration from stem cells in vitro and in vivo, and to discuss the potential for stem cell therapy to replace damaged myocardium and its blood supply in human coronary heart disease.

Stem cells are undifferentiated cells capable of self renewal, proliferation, and differentiation into multiple lineages permitting tissue regeneration.[w2] Haemopoietic stem cells (HSCs) were the first to be identified and exhibit all these properties. A number of types of stem cells are now recognised, as well as partially differentiated progenitor cells that are capable of proliferation and differentiation to multiple lineages.

Embryonic stem cells

Cells isolated from the embryonic blastocyst are capable of proliferating and differentiating into cells of all three embryological germ layers[1] and are capable of differentiating into any cell in the body. Embryonic stem (ES) cells have an inherent tendency to differentiate spontaneously in culture such that specific culture conditions are required to maintain them in their undifferentiated state (fig 1). Nevertheless, under defined conditions they will proliferate indefinitely while retaining their capacity to differentiate, thereby providing a potentially limitless source of cells. Human ES cells were first isolated in 1998 from the inner cell mass of embryos donated by couples who had undergone assisted reproduction.

Tissue specific stem cells

Some mature tissues contain partially differentiated precursor cells that remain quiescent unless stimulated to proliferate and differentiate into mature cells.[w3] However, unlike ES cells, these cells will generally differentiate only into cells contained within their tissue of origin. Thus, for example, skeletal muscle contains satellite cells that will proliferate and mature into adult skeletal myocytes following muscle injury.

Bone marrow derived stem cells

In addition to HSCs, bone marrow contains cells that form the stroma and supporting tissues around HSCs, called bone marrow stromal cells (BMSCs). These cells, also termed mesenchymal stem cells, can differentiate into a variety of non-haemopoeitic cell types including cardiomyocytes[2] and endothelial cells (ECs).[3] BMSCs can be isolated from the circulation, and may contribute to vascular repair after endothelial injury.[w4]

STEM CELLS FOR MYOCARDIAL REPAIR

In order to effect myocardial repair, sometimes termed cellular cardiomyoplasty, stem cell therapy requires delivery of cells either systemically or locally by a number of well established techniques (table 1). Engrafted cells must then proliferate to provide adequate new tissue before differentiating into functional cardiomyocytes that couple mechanically and electrically with the recipient myocardium (fig 2). Depending on their origin, the cells may require considerable manipulation before they are implanted and this, of necessity, will require an understanding of the molecular pathways involved in successful engraftment, survival, and function.

ES cell cardiomyocyte differentiation

Human ES cells in suspension culture, or when injected into adult organisms, spontaneously form embryoid bodies (EBs)[1] containing cells from all three germ layers that exhibit varying degrees of

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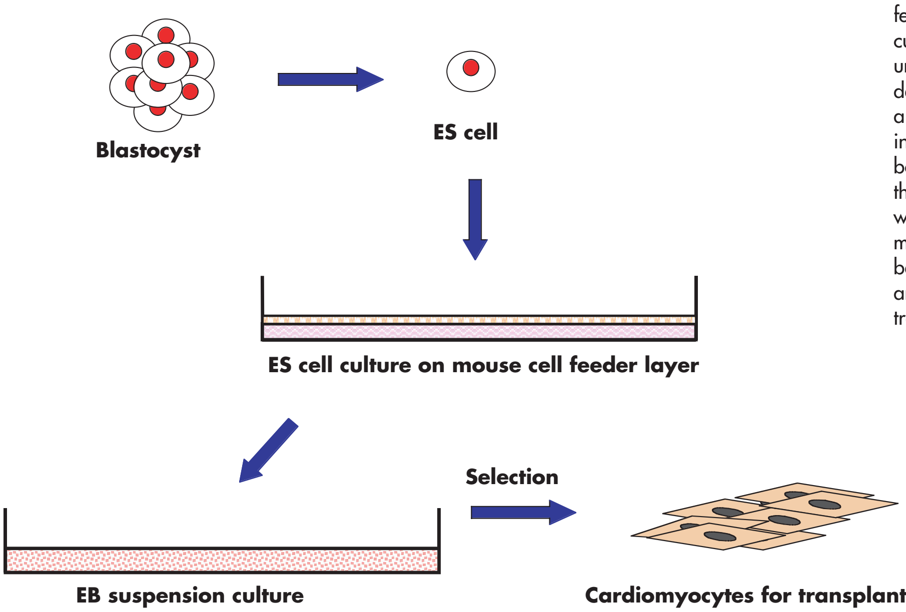

Figure 1 Embryonic stem (ES) cells are derived from the embryological blastocyst at around day 8 after fertilisation. ES cell populations can be cultured and expanded in the undifferentiated state in vitro under defined conditions—for example, using a murine fibroblast feeder layer. ES cells in suspension culture form embryoid bodies (EBs) which contain cells from all three embryological germ layers, some with spontaneously contracting myocardial cells. Cardiomyocytes can be isolated by micro-dissection from EBs and selectively cultured for transplantation.

vascular and cardiac development, with around 8% of EBs containing spontaneously contracting cells.[4] These contracting cells can be isolated from EB outgrowths and exhibit the molecular and electrical characteristics of cardiomyocytes.[w5] ES cells that do not exhibit spontaneous cardiomyogenesis can be stimulated to do so by co-culture with visceral endoderm.[w6] ES cell derived cardiomyocytes appear to be phenotypically identical to fetal cardiomyocytes.[4 w6] However, the precise molecular mediators that drive differentiation of ES cells into cardiomyocytes are currently unknown and are the subject of intense research activity. A number of methods have been used to expand the ES derived cardiomyocyte population in vitro; however, it is currently not possible to induce ES cells to differentiate directly into cardiomyocytes at will.

EB derived, culture enriched cardiomyocytes have been shown to engraft into the myocardium and improve myocardial function in a number of rodent models of heart disease,[5 w7 w8] suggesting that ES cell derived cardiomyocytes could be of therapeutic benefit in human ischaemic heart disease. However, there are a number of practical and ethical concerns which are likely to restrict their use in man.

Because ES cells are derived from embryos that are genetically distinct from the potential recipient they are antigenic. A number of strategies have been proposed to overcome this problem including establishing banks of ES cells representing a wide array of histocompatibility backgrounds,[w9] replacing the patient's haemopoeitic and lymphoid tissue with cells compatible with the ES cells to be transplanted[w10] or rendering the ES cells less antigenic by molecular engineering.[w11] It has also been suggested that only low dose immunosuppressive therapy might be required after transplantation because far fewer antigens are transplanted with a single cell type than with an entire organ.[1] The most promising strategy combines ES cell technology with somatic cell nuclear transfer, in which the nucleus of an oocyte is replaced by the nucleus of an adult cell, such that progeny cells contain the genome of the adult nucleus. The very recent demonstration that a pluripotent ES cell line can be derived with the DNA of adult human cells provides real optimism that it may, in the future, be possible to create autologous ES cells for therapeutic use.[6] However, transplanted, undifferentiated ES cells also have the capacity to form local and disseminated teratomas.[1] This risk can be minimised by employing culture techniques that favour purity of ES cell derived cardiomyocytes and minimise the propagation of undifferentiated ES cells.[4]

ES cells are derived from terminated pregnancies, from residual embryos after assisted fertilisation, and from

Table 1 Techniques for delivery of cells to effect myocardial repair

Route	Delivery method	Comments
Myocardial injection: thoracotomy	Sternotomy, thoracotomy, or VATS	Safety concerns for open surgery. Reduced risk with VATS
Myocardial injection: percutaneous	Injection catheters developed from electrophysiology/PMR equipment	Targeting improved with guidance by left ventricular mapping
Systemic intravenous	Intravenous cannulation	Systemic risks of cellular therapy not known
Intracoronary artery	Cardiac catheter	Targeted cell delivery. Widespread operator experience

PMR, percutaneous myocardial revascularisation; VATS, video assisted thorascopic surgery.

embryos specifically created for research. Therefore their use raises sensitive and complex ethical issues. In the UK, the use of embryological material is strictly regulated by the Human Fertilisation and Embryology Authority. Research which increases knowledge about serious illness or embryo development is permitted up to 14 days after fertilisation. This allows the use of ES cells taken from the blastocyst around day 8.[w12] In contrast, recent legislation in the USA has severely restricted the use of ES cells and in Germany the derivation of new ES cell lines is illegal. The European Parliament has voted to ban ES cell research and therapeutic cloning, although the UK government has stated that it will persist in its stance that ethical issues relating to embryo research should be determined at a national level. The divergence of views around the world has led to efforts to establish an international stem cell consortium to coordinate research, with the expectation that while new ES cell lines will continue to be developed in the UK, research in the US will focus on the in vitro differentiation of existing cell lines.[w13]

Even if ES cell transplantation is not a therapeutic option for the immediate future, it is likely that ongoing research on ES cells will identify the molecular pathways responsible for cardiomyocyte differentiation, thereby enabling manipulation of more acceptable cell sources to effect myocardial repair. Consequently, efforts are being directed towards the use of adult derived stem cells, which may share many of the properties of self renewal, proliferation, and multipotency with ES cells.

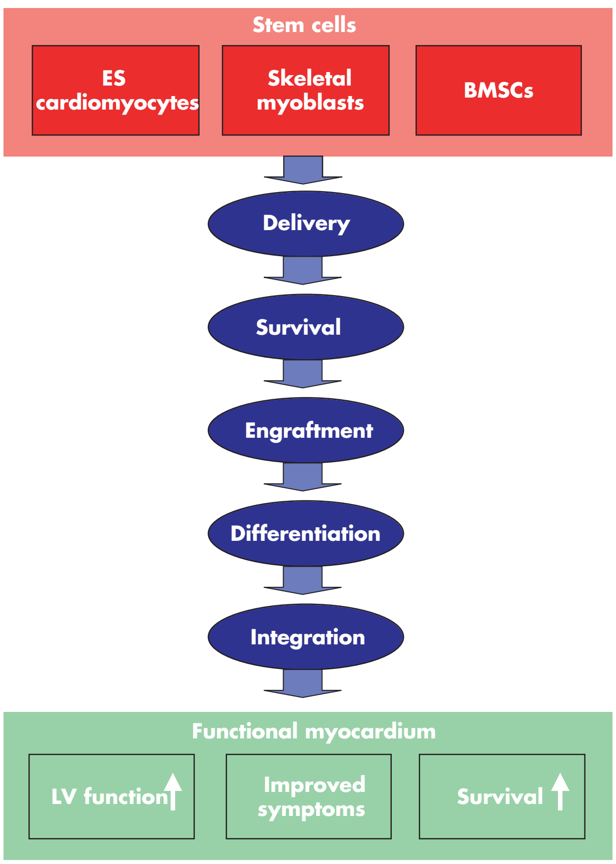

Figure 2 There are a number of steps necessary for successful cardiomyoplasty. After delivery to the target tissue cells must survive and engraft into the host tissue. ES cell derived cardiomyocytes are differentiated before delivery, but skeletal myoblasts and bone marrow stromal cells (BMSCs) must differentiate after transplantation. Integration into the myocardium includes electrical and mechanical coupling with the surrounding myocardium and is subject to a satisfactory vascular supply. The main therapeutic end points are left ventricular (LV) function, symptoms, and survival.

Stem cell properties

- Self renewal
- Proliferation
- Differentiation to multiple lineages

TISSUE SPECIFIC STEM CELLS

Skeletal myoblasts

Adult skeletal muscle contains cells, called myoblasts or satellite cells, that remain quiescent until stimulated, in response to injury, to proliferate and differentiate into mature skeletal myocytes. Myoblasts make up 3–4% of skeletal muscle cells and can be easily isolated and amplified in an undifferentiated state in culture.[7 w14] Because skeletal muscle has a greater tolerance to ischaemia than myocardium, skeletal myoblasts may be well suited for delivery to relatively ischaemic areas of myocardium.[7] Furthermore, autologous transplantation of skeletal myoblasts avoids many of the ethical and immunological considerations associated with ES cells. However, their electrical and mechanical properties differ from those of cardiac myocytes, which may impair their ability to contribute usefully and safely to myocardial function.

Preclinical studies

Skeletal myoblasts have engrafted into both normal and ischaemic myocardium in several animal models with some evidence for functional improvement.[w15 w16] They adopt a slow twitch, striated muscle phenotype with intercalated discs similar to cardiomyocytes, although they do not express all the cellular markers of adult cardiomyocytes[w17] and lack the gap junctions required to transport small molecules and ions between cardiomyocytes, a property that is likely to affect their electrical coupling with host myocardium.[w18] There is also some evidence that myoblast transplantation yields a persistent blast population that may provide some capacity for long term regeneration and repair.[w19]

Clinical studies

Pagani and colleagues injected autologous skeletal myoblasts, cultured from quadriceps muscle biopsies, directly into the failing myocardium of five patients awaiting transplantation at the time of left ventricular assist device implantation.[w20] Hearts from three subjects who underwent transplantation and one who died demonstrated myoblast engraftment, survival, and differentiation to myofibres expressing skeletal myosin heavy chain. The study did not investigate electrical or mechanical coupling. Menasche and colleagues reported improvement in symptoms, left ventricular function, and systolic thickening of previously akinetic segments following injection of autologous skeletal myoblasts directly into the myocardium at the time of coronary artery bypass surgery.[8] However, the contribution of revascularisation to these effects is unclear. Furthermore, four of the 10 patients experienced sustained ventricular arrhythmias that necessitated cardioverter-defibrillator implantation, possibly

Embryonic stem cells

- Derived from the embryonic blastocyst
- Expanded in vitro in an undifferentiated state
- Differentiate to any cell type
- Ethical concerns
- Tissue compatibility and rejection issues

as a result of re-entry circuits established by myoblast engraftment.[8]

These studies show that skeletal myoblasts can be delivered to ischaemic myocardium where they survive and mature and may contribute to contractile performance. However, it remains to be established how long transplanted skeletal myoblasts survive, to what extent they contribute to myocardial function, and how great the risk is of life threatening arrhythmias.

Myocardial stem cells

Despite the generally accepted dogma that adult cardiomyocytes are unable to regenerate, recent research suggests the existence of cardiac stem cells. Studying human hearts shortly after myocardial infarction, Beltrami and colleagues demonstrated a small proportion of cardiomyocytes (0.08%) apparently undergoing mitosis in the peri-infarct tissue.[9] Subsequently, they identified rat heart cells that gave rise, in vitro, to cells expressing cardiomyocyte, EC, and vascular smooth muscle cell (VSMC) markers but did not differentiate into fully mature myocytes and vascular cells.[w21] Nevertheless, when they were labelled with bromodeoxyuridine and injected into infarcted rat myocardium in vivo, they produced new myocardium and new vessels that contributed to improved ventricular contraction. Thus it would appear that there exists, in the rat myocardium at least, a population of relatively undifferentiated cells with the capacity to generate new myocardium and its vasculature. These exciting and, as yet, unconfirmed findings represent a paradigm shift that challenges the dogma that the adult myocardium has no capacity to regenerate and raises expectations that myocardial repair using autologous cells may be achievable. However, several important questions remain unanswered. For example, do these cells arise from the myocardium or do they come from the circulation? Also, if similar cells exist in adult human hearts, why do they not spontaneously repair damaged myocardium? Given the interest generated by these data, these questions should shortly be answered.

BONE MARROW DERIVED STEM CELLS

Bone marrow stromal cells

BMSCs can be isolated from adult bone marrow and trebecular bone and form colonies in culture by clonal proliferation of a single cell. Cells in each colony are capable of differentiating into a wide range of mesodermal tissues (table 2), including bone, cartilage, adipose tissue, fibrous tissue, and marrow stroma both in vitro and in vivo.[10] When BMSCs are transplanted into a blastocyst they are capable of differentiating into cells of all three germ layers, just as do ES cells, suggesting that BMSCs have great potential for stem cell therapy.[w22] Rodent and human BMSCs can be induced in vitro to form contracting myotubes connected by intercalated discs[w23] with gene markers and electrophysiological properties similar to those of adult cardiomyocytes.[2 w24] Thus autologous BMSCs are accessible and might have the capacity to effect myocardial repair. Furthermore, it has recently been shown that stromal stem cells isolated from adipose tissue can also differentiate into cardiomyocytes,[w25] offering a promising source of cardiomyocyte precursors.

Preclinical studies with BMSC

A key, promising observation has been that undifferentiated mesenchymal stem cells can be transplanted, without manipulation, into adult tissues where the local environment apparently provides sufficient chemical and cellular stimuli to trigger differentiation. In a mouse model, BMSCs injected into infarcted myocardium adopted a cardiomyocyte phenotype and formed intercalated discs with host cells.[11] The levels of engraftment were low, but there were measurable improvements in ventricular function, reduction in infarct size, and improved survival. Donor marrow cells also incorporated into blood vessels, where they adopted both EC and VSMC phenotypes, although it is not clear whether they contributed to neovascularisation. Further work by this group has demonstrated that bone marrow derived cells can form up to 68% of new myocardium when injected into the myocardial infarct border in a mouse coronary artery ligation model.[w26] In a rat model of AMI, autologous BMSCs labelled with a LacZ reporter gene and delivered by coronary artery injection were identified at eight weeks within and around the infarcted myocardium.[w27] The transplanted cells expressed cardiomyocyte markers and some had formed gap junctions with endogenous cardiomyocytes. There was also evidence for improved left ventricular function.

Human BMSCs, cultured from bone marrow aspirates from healthy volunteers (fig 3), showed a similar response when injected into rat myocardium,[12] but only a limited number of cells survived beyond seven days; although the human BMSCs appeared to engraft and expressed a number of

Table 2 Types of cells derived from the different cell fractions

Cell fraction	Cells derived
Embryonic stem cell	All cell types
Bone marrow stromal cell	Adipocyte Osteoblast Vascular smooth muscle cell Cardiomyocyte
Bone marrow mononuclear cell	Adipocyte Osteoblast Vascular smooth muscle cell Cardiomyocyte Endothelial cell
Endothelial progenitor cell	Endothelial cell Haemopoietic cells
Endothelial outgrowth cell	Endothelial cell

Adult stem cells

- Quiescent in adult tissues
- Stimulated to divide for tissue regeneration
- Differentiate to a limited range of cell types
- Potential for autologous transplantation
- Differentiate in response to host stimuli after transplantation

cardiomyocyte markers, they did not express key proteins such as troponin T.

Based on the evidence available so far, therefore, BMSCs appear potentially to have all the properties required of an autologous stem cell population that might be able to repair the heart. However, a recent study in dogs found that injections of mesenchymal stromal cells into the coronary arteries of healthy dogs caused microscopic and macroscopic myocardial infarction.[w28] Furthermore, as the field has progressed, it has become clear that that the presence of BMSC markers in an apparently mature myocyte does not necessarily mean that the BMSC has differentiated into a myocyte. BMSCs may fuse with resident cells to create a cell that expresses markers of both cell types, thereby giving the false impression of successful engraftment and differentiation. Observations such as these reflect the relative infancy of this field of science and point towards a cautious approach to its clinical application.

Clinical studies with BMSCs

Evidence that circulating cells may engraft human hearts comes from observations in heart transplantation where recipient cardiomyocytes have been identified in the donor myocardium of sex mismatched transplants.[13 14] Therefore attempts have been made to deliver BMSCs to the heart via the circulation. Strauer and colleagues infused a mixed population of autologous bone marrow cells that included BMSCs, endothelial progenitors, and monocytes, collectively called bone marrow mononuclear cells (BMMCs), into the infarct related coronary arteries of 10 patients within 72 hours of their infarct. Compared with 10 patients who received conventional treatment, those who received BMMCs had apparently smaller infarcts and better myocardial performance three months after treatment.[15] In the TOPCARE-AMI (transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction) study myocardial infarction patients received endothelial progenitors cells (EPCs) or BMMCs via the infarct related artery after reperfusion and appeared to derive significant benefits in measures of infarct size and left ventricular performance.[16 17]

These results are encouraging and appear to confirm the safety of this therapeutic approach. However, since neither study had a suitable placebo treated control group, it is too early to draw any conclusions on its efficacy. Furthermore, neither study was designed to provide information on the fate of the infused cells or how they contributed to the observed benefits. Further, carefully controlled studies are

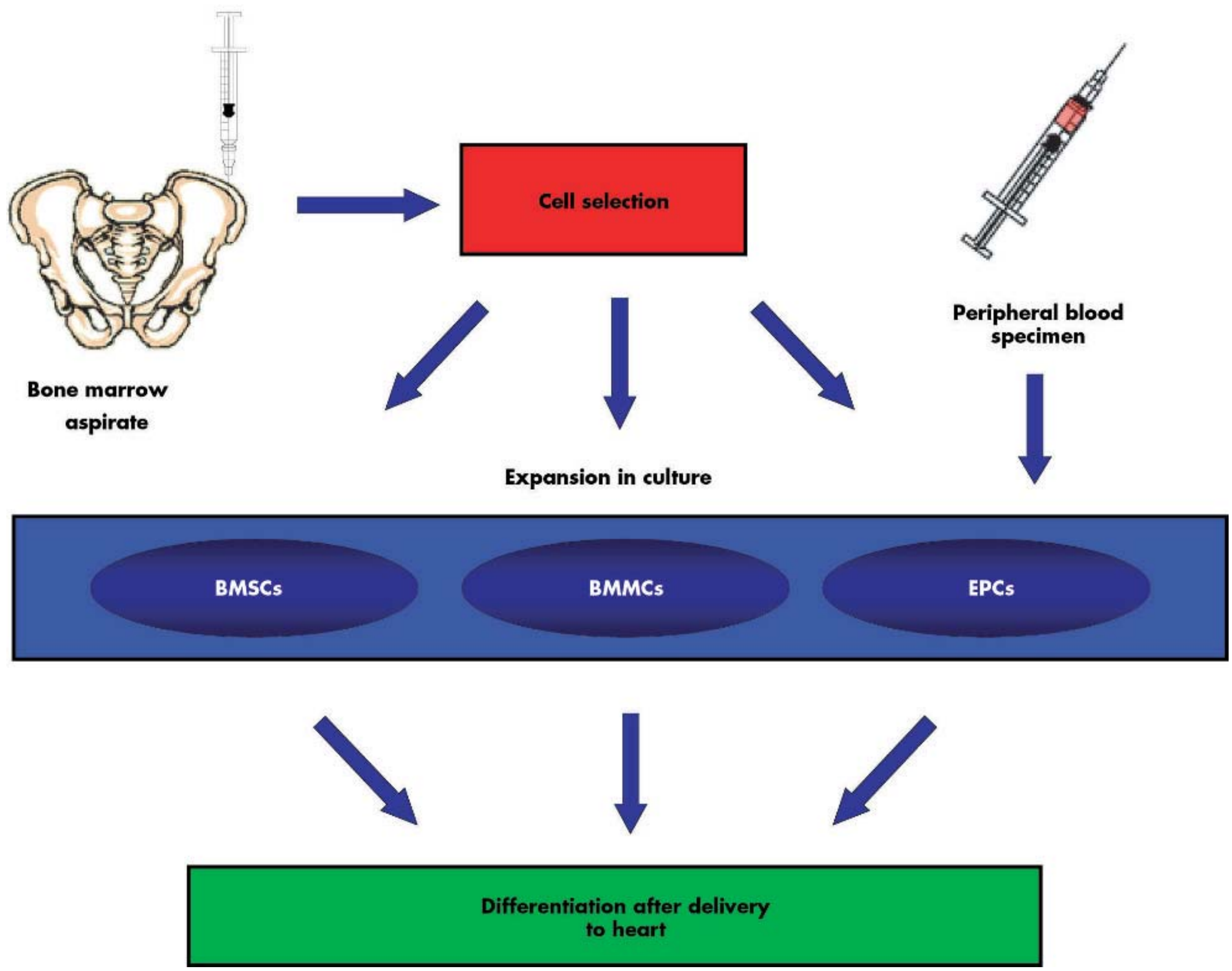

Figure 3 A number of cell populations can be isolated from bone marrow. Bone marrow stromal cells (BMSCs), bone marrow mononuclear cells (BMMCs), and endothelial progenitor cells (EPCs) separated in this way can be expanded in culture for autologous transplantation. Circulating EPCs can also be isolated and expanded in culture. These cell populations undergo differentiation after delivery into the target tissue.

required to determine if transplantation of bone marrow derived cells can regenerate damaged myocardium and, if so, by what mechanism.

STEM CELLS FOR VASCULAR REPAIR

The fundamental problem in ischaemic heart failure is an inadequate blood supply, and even if new myocytes can be generated, they will require a mature, integrated vascular system if they are to survive and function. It is currently unclear whether cellular therapy to provide new myocardium will initiate sufficient host angiogenesis to provide an adequate blood supply, or whether additional therapy will be needed to achieve this.

Entirely new blood vessel formation begins with vasculogenesis, the formation of blood islands from haemangioblasts, the common precursor to ECs and blood cells,[w29] whereas angiogenesis refers to the formation of a new vascular network as a result of sprouting, bridging, and intussusception of existing vessels. New EC tubes recruit local mesenchymal cells to differentiate into pericytes and VSMCs, a process known as arteriogenesis. Failure of arteriogenesis results in leaky and poorly functioning EC tubes. A good example of this is diabetic retinopathy where immature new vessels form in response to local ischaemia. Development of a mature vascular system involves the coordinated expression of multiple angiogenic factors, and the complete repertoire of molecular signals and angiogenic factors required to create a mature vascular system are currently unknown. It is therefore not surprising that early attempts to improve myocardial perfusion by the delivery or overexpression of single angiogenic factors have had limited success.[w30–32] An alternative approach is to introduce vascular precursor cells with the capacity to orchestrate the formation of new mature blood vessels.

Circulating endothelial precursors

Endothelial cells and blood cells share a common precursor, termed a haemangioblast, and it has been found that some HSCs can differentiate to express endothelial markers.[3] EPCs have been isolated from human bone marrow aspirates and have also been identified in the circulation. They are mobilised in response to tissue ischaemia and endothelial injury and can differentiate into mature ECs.[18] There is convincing experimental evidence that EPCs can incorporate into ischaemic myocardium and differentiate to ECs that contribute to improved perfusion and function.[19 20] This raises the possibility of harvesting autologous EPCs for human therapy either as vascular precursors themselves or as cellular vectors for gene therapy. For example, adenoviral transduction of EPCs with vascular endothelial growth factor (VEGF) increased their proliferation in vitro, and improved their neovascularisation potential in a mouse model of hind limb ischaemia.[21]

Bone marrow derived stem cells have been used in an attempt to improve myocardial perfusion in man. In the MAGIC cell study, CD34+ stem cells were harvested after treatment with granulocyte colony stimulating factor (G-CSF) and were injected into a coronary artery during stenting after myocardial infarction. Patients treated with the stem cells showed improved perfusion and left ventricular systolic function at six months.[22] However, there was also increased in-stent restenosis that was ascribed to the mitogenic effects of G-CSF. As with other clinical studies described above, this study was small and unable to determine the fate of the infused cells or their contribution to the observed effects.

SUMMARY

The ability to create new, perfused myocardium to replace that damaged by ischaemia appears tantalisingly close. ES cells clearly have the capacity to differentiate into adult myocytes and vascular cells, but ethical and logistic hurdles are likely to prevent their widespread therapeutic use for the foreseeable future. Nevertheless, it is only a matter of time before research on ES and other stem cells reveals the precise molecular mechanisms involved in cardiomyocyte and vascular differentiation, thereby paving the way for the exploitation of more readily accessible and ethically acceptable autologous cells, such as those derived from bone marrow, for therapeutic purposes. Animal studies of cellular therapy for coronary heart disease have confirmed the potential for cardiomyogenesis and neovascularisation, and clinical trials with autologous skeletal myoblasts and BMSCs have demonstrated the feasibility of the stem cell approach to treatment in man and have indicated potential complications.

However, despite optimism generated by these promising early clinical studies, there is currently no robust evidence that autologous cells can survive, engraft, differentiate, and function appropriately in the human heart. The most optimistic message comes from the identification of resident rat myocardial stem cells that can differentiate into all the cell types required to create a new myocardium in an apparently controlled and coordinated manner. Confirmation of a similar process in the human heart would represent a very major positive step towards realising the goal of myocardial repair.

It is essential that myocardial repair strategies are subjected to rigorously controlled clinical trials to determine their effects on clinical outcomes and to confirm that any observed effects are indeed due to engraftment and differentiation of the administered cells. Recent advances in cellular and molecular imaging using magnetic resonance imaging and positron emission tomography may provide the tools for such studies. Failure to do so may lead to yet another false dawn on the cardiological horizon.

Additional references appear on the *Heart* website—http://heartjnl.com/supplemental

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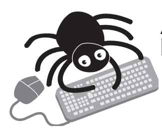

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